CRYPTOCHROME Is a Blue-Light Sensor That Regulates Neuronal Firing Rate

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(American Geophysical Union, Washington, DC, 2003), Geophysical Monograph vol. 136, pp. 241–254.
28. W. S. Holbrook, P. B. Kelemen, *Nature* **364**, 433 (1993).
29. Detailed methodology and all original analytical data are available as supporting material on *Science* Online.
30. T. E. Cerling, in *Palaeoweathering, Palaeosurfaces and Related Continental Deposits*, M. Thiry, R. Simon-Coinçon, Eds. (Special Publications of the International Association of Sedimentologists, Oxford, 1999), vol. 27, pp. 43–60.
31. J. Quade, T. E. Cerling, J. R. Bowman, *Geol. Soc. Am. Bull.* **101**, 464 (1989).
32. N. C. Arens, A. H. Jahren, R. Amundson, *Paleobiology* **26**, 137 (2000).
33. D. O. Breecker, Z. D. Sharp, L. D. McFadden, *Geol. Soc. Am. Bull.* **121**, 630 (2009).
34. J. H. Whiteside, P. E. Olsen, T. Eglinton, M. E. Brookfield, R. N. Sambrotto, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 6721 (2010).
35. R. K. Suchecki, J. F. Hubert, C. C. B. De Wet, *J. Sediment. Petrol.* **58**, 801 (1988).
36. D. M. Cleveland, S. C. Atchley, L. C. Nordt, *J. Sediment. Res.* **77**, 909 (2007).
37. S. I. Dworkin, L. Nordt, S. Atchley, *Earth Planet. Sci. Lett.* **237**, 56 (2005).
38. D. Beerling, *Nature* **415**, 386 (2002).
39. G. J. Retallack, *Nature* **415**, 387 (2002).
40. L. H. Tanner, *The Great Rift Valleys of Pangea in Eastern North America*, P. M. L. Tourneau, P. E. Olsen, Eds. (Columbia Univ. Press, New York, 2003).
41. J. H. Whiteside, P. E. Olsen, D. V. Kent, S. J. Fowell, M. Et-Touhami, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **244**, 345 (2007).
42. D. J. Beerling, D. L. Royer, *Annu. Rev. Earth Planet. Sci.* **30**, 527 (2002).
43. G. J. Retallack, *Nature* **411**, 287 (2001).
44. P. E. Olsen, *U.S. Geol. Surv. Bull.* **1776**, 6 (1988).
45. H. Svensen *et al.*, *Nature* **429**, 542 (2004).
46. J. C. G. Walker, P. B. Hays, J. F. Kasting, *J. Geophys. Res.* **86**, 9776 (1981).
47. C. Dessert, B. Dupre, J. Gaillardet, L. M. Francois, C. J. Allegre, *Chem. Geol.* **202**, 257 (2003).
48. A. S. Cohen, A. L. Coe, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **244**, 374 (2007).
49. M. S. Fedosh, J. P. Smoot, *U.S. Geol. Surv. Bull.* **1776**, 19 (1988).
50. R. A. Berner, Z. Kothavala, *Am. J. Sci.* **301**, 182 (2001).
51. D. V. Kent, L. B. Clemmensen, *Bull. Geol. Soc. Den.* **42**, 121 (1996).
52. We are grateful to J. Quade and P. E. Olsen for their numerous helpful discussions and L. Godfrey for her technical lab assistance. This research was supported by 2009 Geological Society of America and the Society of Economic Mineralogists and Petrologists Graduate Research Awards and NSF grant EAR 0958867. This work is Lamont-Doherty Earth Observatory Contribution #7432.

Supporting Online Material

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CRYPTOCHROME Is a Blue-Light Sensor That Regulates Neuronal Firing Rate

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Light-responsive neural activity in central brain neurons is generally conveyed through opsin-based signaling from external photoreceptors. Large lateral ventral arousal neurons (lLNvs) in *Drosophila melanogaster* increase action potential firing within seconds in response to light in the absence of all opsin-based photoreceptors. Light-evoked changes in membrane resting potential occur in about 100 milliseconds. The light response is selective for blue wavelengths corresponding to the spectral sensitivity of CRYPTOCHROME (CRY). *cry*-null lines are light-unresponsive, but restored CRY expression in the lLNv rescues responsiveness. Furthermore, expression of CRY in neurons that are normally unresponsive to light confers responsiveness. The CRY-mediated light response requires a flavin redox-based mechanism and depends on potassium channel conductance, but is independent of the classical circadian CRY-TIMELESS interaction.

The *Drosophila melanogaster* circadian clock circuit is composed of 140 to 150 neurons in the central brain and includes PIGMENT-DISPERSING FACTOR (PDF)–expressing lateral ventral neurons. The large lateral ventral neurons (lLNvs) are arousal neurons (*1*–*3*) and increase spontaneous action potential firing in response to light (*4*), whereas the small lateral ventral neurons (sLNvs) are critical for circadian function (*5*). Light resets the circadian clock via two mechanisms (*6*): rhodopsin-based external photoreceptors [the compound eye, ocelli, and the Hofbauer-Buchner (HB) eyelet] and the blue-light photopigment CRYPTOCHROME (CRY). *Drosophila* CRY is best known for its light-activated targeting of TIMELESS (TIM) for degradation, resetting the clock (*7*–*9*). External photoreceptors and CRY entrain the *Drosophila* circadian circuit at vanishingly low light levels (*10*, *11*). CRY also mediates magnetosensitivity in flies and butterflies (*11*–*13*).

In addition to the circadian molecular clock, membrane excitability is a key component of normal maintenance of circadian rhythms (*14*). Electrophysiological characterization of the s- and lLNvs has shown that their membrane properties are circadian-regulated outputs as well. Spontaneous firing frequencies are higher during the early day, gradually drop until dusk, and then rise again through the course of the night (*1*, *15*). Additionally, the lLNv spontaneous firing frequency elevates 20 to 200% in response to moderately bright light (*4*). Given the plurality of light inputs to the lLNv, we investigated the lLNv electrophysiological light response (*16*) and found that the response is due to CRY acting by a cell-autonomous, redox-based mechanism, independent of CRY-TIM interactions, which requires the conductance of membrane potassium channels. Furthermore, ectopic expression of CRY optogenetically confers electrophysiological light responsiveness to neurons that ordinarily do not respond to light.

Results. Both tonic and burst firing lLNvs recorded in the whole-cell current clamp configuration in an acutely dissected whole-brain preparation from flies expressing the *pdfGAL4* driver and green fluorescent protein (GFP)–tagged nonconducting UAS-dORK, a *Drosophila* membrane-delimited potassium channel (*4*, *14*), (*pdfGAL4-NC1-GFP*) under dark conditions (>0.02 mW/cm^2) immediately increased their firing rate and their resting membrane potential in response to moderate-intensity white light (4 mW/cm^2) (Fig. 1A, top) or high-intensity blue light (19 mW/cm^2) from a mercury light source (450 to 490 nm) (Fig. 1A, bottom), then rapidly returned to baseline firing rate upon return to darkness. The strength of the firing frequency lLNv light response, expressed here as the firing frequency with the lights on divided by the firing frequency with the lights off (FF on/FF off), varied with light intensity, exhibiting significantly higher firing frequency during lights on compared with lights off at intensities of 2 to 3 mW/cm^2 or higher (Fig. 1B). FF on/off for 19 mW/cm^2 was 1.62 ± 0.14 (n = 11) for 4 to 5 mW/cm^2 was 1.51 ± 0.15 (n = 18), for 2 to 3 mW/cm^2 was 1.39 ± 0.06 (n = 68), for 1 to 2 mW/cm^2 was 1.18 ± 0.02 (n = 27), for 0.6 mW/cm^2 was 1.23 ± 0.06 (n = 16), and for 0.3 mW/cm^2 was 1.10 ± 0.04 (n = 13). Light responses to intensities of 19 mW/cm^2, 4 to 5 mW/cm^2, and 2 to 3 mW/cm^2 were significantly different from 1 to 2 mW/cm^2 [P = < 0.0001, 0.006, and 0.02, respectively, by analysis of variance (ANOVA)].

The lLNvs anatomically appear to receive input from the compound eyes and the HB eyelet. To determine whether the lLNv light response is due to synaptic inputs from external opsin-based photoreceptors, we recorded lLNv in *glass60j* (*gl60j*) mutant flies, which lack all external photoreceptors because of a null mutation in the *eyeless* gene (*6*). The lLNv response to moderate-intensity white light for *gl60j* flies was 1.37 ± 0.09 (n = 14; P = 0.81

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versus control); under intense blue light, the response of *gl60j* flies was 1.59 ± 0.1 (n = 5, P = 0.82 versus control, as tested by ANOVA). Thus, the responses to white and intense blue light do not differ between control and *gl60j* mutant flies (Fig. 1C).

The intact lLNv light response in *gl60j* mutant flies suggests that the blue-light photopigment CRY expressed in the lLNv may underlie the response. *Drosophila* CRY is excited maximally at 450 nm and absorbs wavelengths no longer than 530 nm (*17*), so the lLNv response to discrete wavelength ranges was tested. The lLNvs significantly increased their firing rate in response to both moderate intensity blue-green light (<550 nm; 1.34 ± 0.04, n = 26, P << 0.001 by paired t test of lights on versus off) and low-intensity blue-violet light (375 to 450 nm; 1.33 ± 0.02, n = 70, P << 0.001), but not to red-orange light (>550 nm; 1.04 ± 0.02, n = 23, P = 0.18) (Fig. 1, D and E). The spectral profile of the lLNv light response does not differ when tested in *gl60j* mutant flies (Fig. 1E, FF on/off is 1.37 ± 0.09, n = 14, P = 0.001 for white light; 0.94 ± 0.05, n = 9, P = 0.54 for orange-red light; 1.21 ± 0.07, n = 12, P = 0.003 for blue-green; 1.29 ± 0.05, n = 14, P < 0.001 for blue-violet light.) For comparisons for a given wavelength range between control and *gl60j*, P values were 0.81, 0.42, 0.27, and 0.64 for white, orange, blue-green, and blue-violet light, respectively. The lack of lLNv responsiveness to wavelengths > 550 nm shows that infrared does not contribute to light-driven increases in firing frequency. The spectral profile of the lLNv light response matches that of CRY but requires about five orders of magnitude higher light intensity than that required for CRY's known role in resetting the circadian clock via TIM degradation and occur between four to five orders more rapidly than the first biochemical indications of CRY-mediated TIM degradation. Last, unlike CRY-mediated TIM degradation, the lLNv light response is reversible, suggesting that the two CRY-mediated phenomena occur via distinct mechanisms.

Hypomorphic cry^b mutant flies, which exhibit impaired circadian function because of a point mutation in the flavin chromophore binding site, display a weakened lLNv response to white light compared with that of their control counterparts (*4*). We quantified the light response of cry^b lLNvs and tested its spectral properties. Figure 1E (right) shows that, like in control and *gl60j* flies, white, blue-green, and blue-violet wavelengths evoke a significant increase in firing frequency (FF on/off = 1.14 ± 0.01 for white light; 1.13 ± 0.02 for blue-green light; 1.16 ± 0.03 for blue-violet light; P < 0.01 by paired t test in each case) in cry^b mutant flies' lLNvs. However, these cry^b light responses are significantly smaller than for their corresponding wavelengths in control flies (P = 0.02 for white, P = 0.005 for blue-green, and P = 0.004 for blue-violet by ANOVA).

To determine whether CRY is necessary for the lLNv light response, we recorded lLNvs from two CRY-null lines, cry^{01} and cry^{02}. Immunocytochemistry confirms baseline native CRY expression in control flies (Fig. 2A), replicating previous results with this CRY antisera (*18*), and no staining of CRY-positive neurons in either CRY null line (Fig. 2B); however, CRY expression was selectively rescued in the LNvs in both CRY null lines when UAS-CRY was driven by *pdfGAL4* (Fig. 2C). The lLNvs of the cry^{01} and cry^{02} null flies exhibited normal spontaneous firing but no light response (Fig. 2, D, top, and E) (cry^{01} response = 1.03 ± 0.04, n = 26, P = 0.007 versus control; cry^{02} response = 1.04 ± 0.03, n = 21; P = 0.01 versus control, ANOVA), indicating that CRY is required for the lLNv light response. The lLNv light response was restored to levels indistinguishable from controls by LNv CRY expression in both cry^{01} (FF on/off = 1.37 ± 0.15, n = 17, P = 0.99 versus control) and cry^{02} (FF on/off = 1.48 ± 0.10, n = 14, P = 0.98 versus control; Fig. 2, D, lower panels, and E) genetic background flies. Although the PDF driver does target the sLNvs in addition to the lLNvs, we have observed light sensitivity in the lLNvs of flies whose sLNvs have been genetically ablated (fig. S1) [also (*1*)]. Thus, we conclude that the CRY-driven lLNv light response is cell-autonomous and independent of CRY expression in external photoreceptors or other central brain neurons.

To determine whether CRY can optogenetically confer electrophysiological light responsiveness to inherently light-insensitive neurons, we targeted CRY expression to olfactory projec-

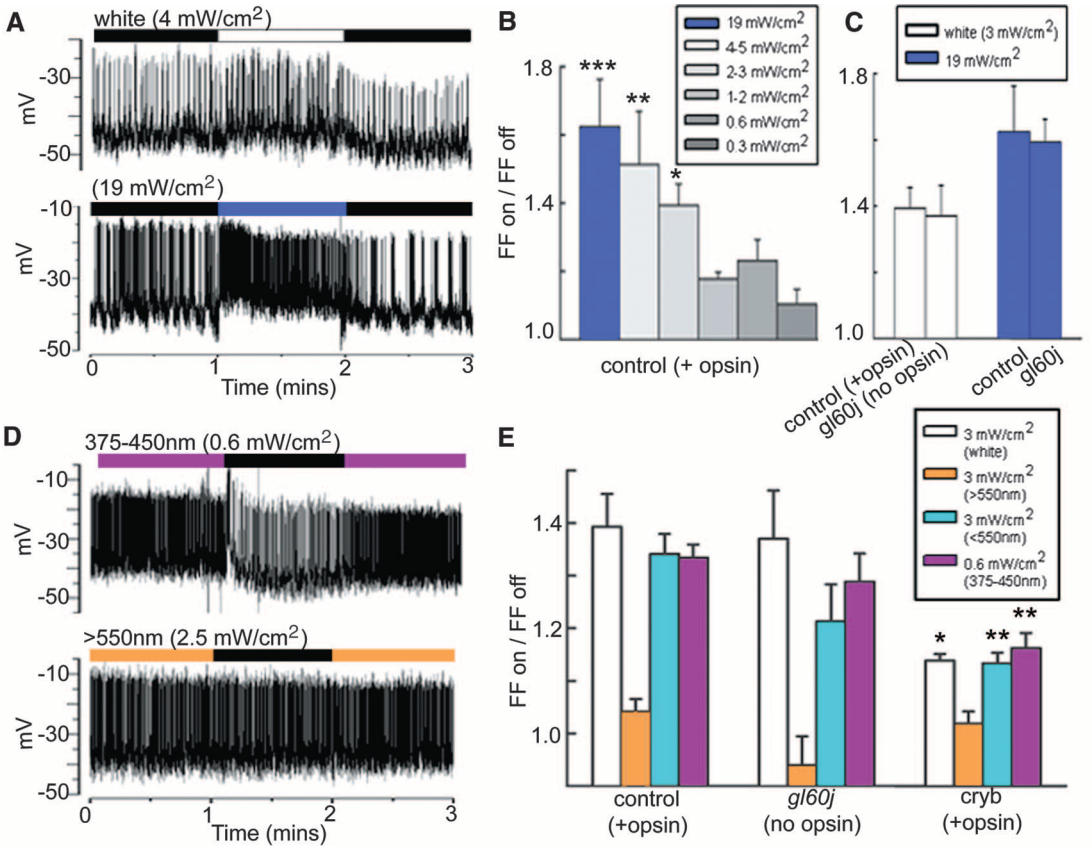

Fig. 1. *Drosophila* lLNvs rapidly increase spontaneous action potential firing rate in response to light independent of opsin-based classical photoreceptors. (**A**) (Top) Response of a representative tonic firing cell to 4 mW/cm^2 halogen white light. (Bottom) Light response of burst firing cell to 19 mW/cm^2 blue light. Alternating light/dark cycles denoted by white or blue versus black bars above traces. (**B**) Firing frequency in light/dark varies according to light intensity. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.005$; error bars indicate SEM. (**C**) Genetic ablation of all external opsin-based photoreceptors has no effect on the lLNv light response. (**D**) Representative recordings of lLNv light response evoked by blue-violet and orange-red light. Alternating light/dark cycles denoted by violet, orange, and black bars. (**E**) Spectral profiles of light responses of lLNv from control versus *eyeless*-null *gl60j* mutant flies are indistinguishable, but responses to white and blue light of cry^b hypomorphs are significantly reduced.

tion neurons with the *GH146-GAL4* driver (fig. S2). This is a rigorous test of (i) CRY's ability to autonomously confer light responsiveness, (ii) a conserved mechanism for coupling CRY light activation to membrane changes in nonclock neurons, and (iii) independence of CRY/TIM interaction. CRY-expressing olfactory neurons increased firing rate recorded in voltage clamp mode in response to intense blue light (Fig. 3A), whereas control CRY-minus olfactory neurons were nonresponsive to white (FF on/off = 1.04 ± 0.02, n = 8), blue-green (0.99 ± 0.04, n = 8), blue-violet (1.01 ± 0.03, n = 8), intense blue (1.04 ± 0.03, n = 8), and orange-red (0.96 ± 0.06, n = 8) light (Fig. 3B, left cluster of bars). The spectral profile of the light response of the CRY-expressing (*GH146-GAL4/UAS-CRY*) olfactory projection cells is almost identical to CRY-positive lLNvs (Fig. 3B, right) responding to white (FF on/off = 1.14 ± 0.02, n = 22), blue-green (1.27 ± 0.04, n = 10), blue-violet (1.18 ± 0.04, n = 18), and intense blue light (19 mW/cm^2 to 35 mW/cm^2, 1.47 ± 0.09, n = 10), but not to orange-red light (1.03 ± 0.03, n = 10). The light response of the CRY-expressing olfactory neurons was significantly higher for blue-green (P < 0.0001), blue-violet (P < 0.005), and intense blue light (P < 0.0001, one-way ANOVA) than counterparts measured in control (non–CRY-expressing) cells.

To probe the mechanism of the CRY-mediated light response, we assessed the ability of different CRY isoforms expressed in the LNv to rescue the light response in CRY-null flies. The *cry*^{m} mutant (a nine–amino acid C-terminal truncation of CRY) binds equally well to TIM in light and dark (*19*), whereas wild-type CRY interaction with TIM is light-dependent. This allowed us to ask whether a light-dependent CRY-TIM interaction is necessary for the acute light response of the lLNvs. The firing rate of CRY^M-expressing lLNv increased significantly in response to white (FF on/off = 1.19 ± 0.05, n = 14) or blue-violet (1.28 ± 0.06, n = 12, P = 0.20 versus white light) light, but not to red-orange light (1.00 ± 0.05, n = 9, P = 0.005 versus white light) (Fig. 4, A and B). Significant large light responses were recorded in the lLNvs in *tim*-null flies for moderate-

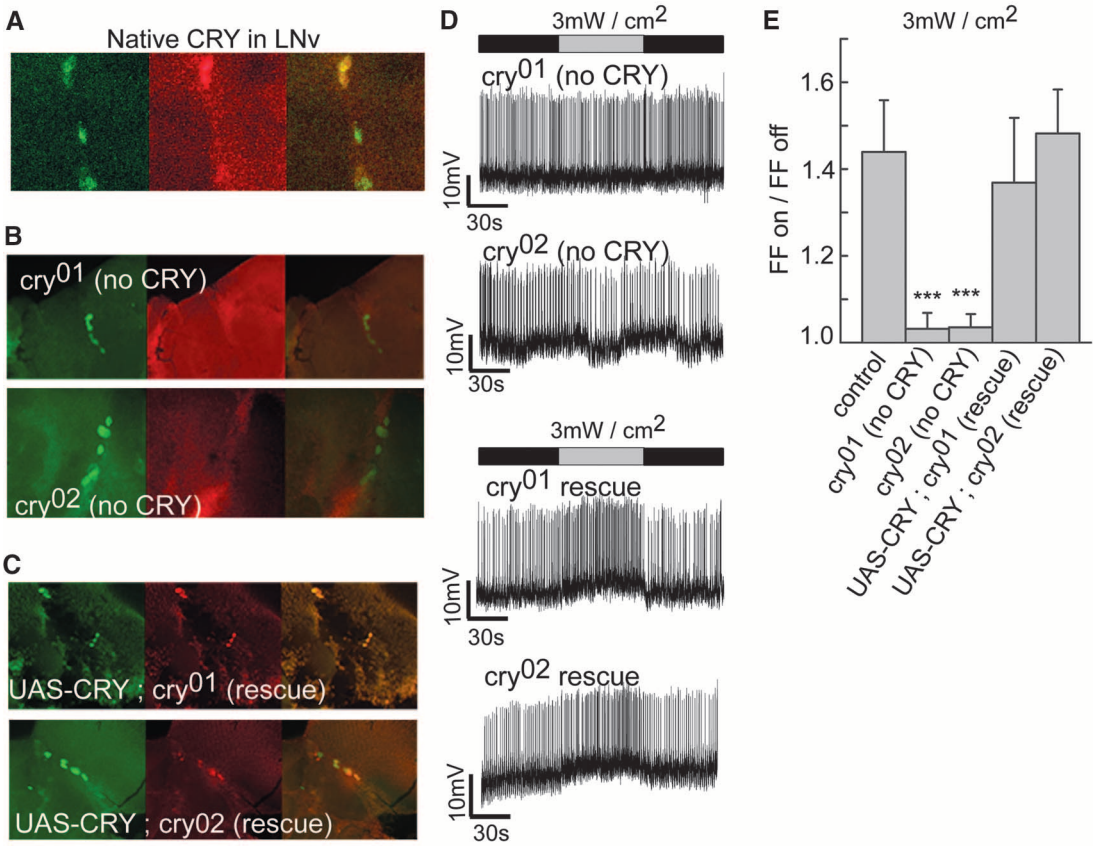

Fig. 2. The lLNv light response is absent in *cry*-null flies but is functionally rescued by targeted expression of CRY in the LNvs. (**A**) Native CRY is detected by anti-CRY and colocalizes in the LNv to a *cryGAL4*-driven GFP signal. (**B**) Anti-CRY signal (red, middle) is absent in LNv (green *pdfGAL4/UAS-dORK-NC1-GFP*, left) in *cry*^{01} and *cry*^{02} null flies (overlay, right). (**C**) Verification of anti-CRY signal (red) expressed specifically in LNv (labeled in green, left, *pdfGAL4/UAS-dORK-NC1-GFP*) in *cry*^{01} and *cry*^{02} null flies that express CRY driven by *pdfGAL4* (overlay, right). (**D**) Representative recordings of *cry*-null flies (top) and genetic rescue of the lLNv light response in *cry*-null flies (bottom). (**E**) lLNv FF response evoked by 3 mW/cm^2 white light/dark does not differ between control and LNv specific expression of CRY in *cry*^{01} and *cry*^{02} null genetic background flies.

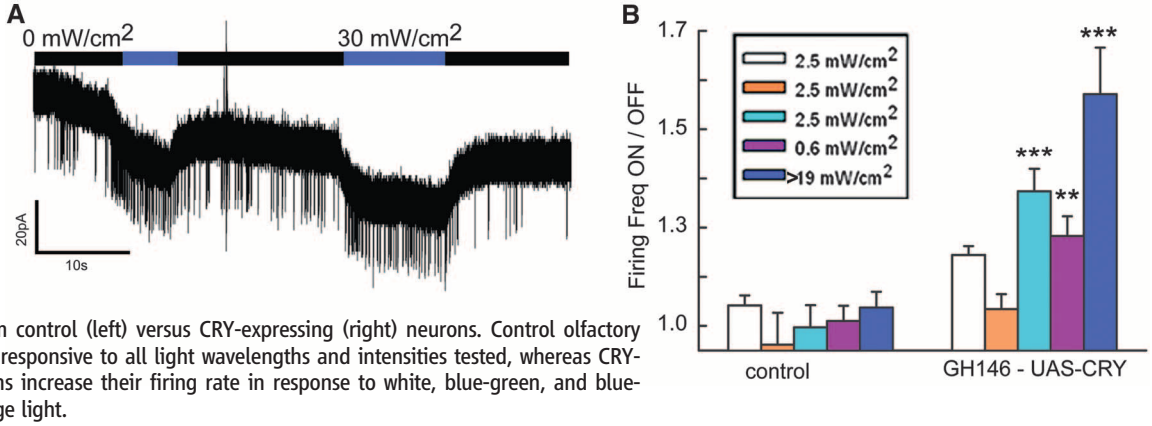

Fig. 3. Ectopic expression of CRY in inherently light-insensitive neurons renders them light-responsive. (**A**) Representative voltage clamp recording of CRY-expressing olfactory projection neuron shows light response evoked by 30 mW/cm^2 blue light. (**B**) Spectral profile of light responses of olfactory projection neurons recorded from control (left) versus CRY-expressing (right) neurons. Control olfactory projection neurons are nonresponsive to all light wavelengths and intensities tested, whereas CRY-expressing olfactory neurons increase their firing rate in response to white, blue-green, and blue-violet light but not to orange light.

intensity white (FF on/off = 1.24 ± 0.03, n = 14) and low-intensity blue-violet light (1.25 ± 0.04, n = 14, P = 0.89 versus white light), but not for moderate-intensity orange-red light (1.00 ± 0.02, n = 14, p = 0.01 versus white light) (Fig. 4C). Thus, TIM interaction is not necessary for the CRY-mediated lLNv light response.

In addition to having the *Drosophila*-like light-responsive CRY1, many insects express a second, vertebrate-like CRY2 that is a potent transcriptional regulator but lacks light sensitivity (*20*). Because *Drosophila* CRY may also act as a transcriptional regulator (*21*), we recorded lLNvs expressing either monarch butterfly (*Danaus plexippus*) dpCRY1 or dpCRY2 in the LNvs of cry^{01} flies. The lLNvs expressing dpCRY1 were electrophysiologically responsive to moderate-intensity white (FF on/off = 1.19 ± 0.05, n = 15) and low-intensity blue-violet (1.16 ± 0.04, n = 14, P = 0.27 versus white light) light but not to moderate-intensity orange-red light (1.01 ± 0.05, n = 10, P = 0.007 versus white light, ANOVA) (Fig. 4, B and C). In contrast, l-LNvs expressing dpCRY2 showed no significant response to white (FF on/off = 1.06 ± 0.04, n = 12), blue-violet (1.02 ± 0.04, n = 14, P = 0.78 versus white), or orange-red (1.02 ± 0.06, n = 8, P = 0.86 versus white) light (Fig. 4, B and C). Paired t tests comparing firing frequency in light versus dark showed no significant response for any wavelength for dpCRY2 (P = 0.62 for white, 0.23 for blue-violet, and 0.74 for orange-red light). Thus, the light response requires CRY that is light-sensitive but not transcriptionally active.

Because a point mutation destabilizes the flavin chromophore binding site in cry^{b} mutants (*9*), we asked whether acute inhibition of the light-activated flavin redox reaction of CRY blocks the lLNv light response. The lLNv respond to blue-violet light at baseline (Fig. 4, D and E, FF on/off = 1.37 ± 0.08, n = 13). Within 5 min of bath-applied flavin-specific redox inhibitor diphenyleneiodonium chloride (DPI), the response was attenuated (FF on/off = 1.10 ± 0.06, n = 5, P = 0.16 versus 5-min sham perfusion). Within 10 min of DPI exposure, blue-violet light no longer evoked a light response (FF on/off = 1.06 ± 0.06, n = 10, P = 0.0008 for 10 min; 1.04 ± 0.08, n = 11, P = 0.01 for 20 min; 1.06 ± 0.04, n = 13, P = 0.0008 for 30 min; all comparisons by ANOVA versus same time in sham perfusion). In contrast, the lLNv light response in vehicle-treated controls was stable over 30 min (FF on/off = 1.39 ± 0.07 and n = 10 at zero; 1.27 ± 0.08, n = 6 at 5 min; 1.42 ± 0.10, n = 10 at 10 min; 1.30 ± 0.06, n = 10 at 20 min; and 1.36 ± 0.06, n = 11 at 30 min; P > 0.31 all time points compared by ANOVA) (Fig. 4D). Note that DPI does not alter spontaneous baseline firing frequency recorded from the lLNv for up to 30 min, indicating the absence of nonspecific metabolic effects on firing (Fig. 4E).

Light-activation of the flavin chromophore of CRY appears to couple to depolarization of neuronal membrane potential, resulting in increased firing rate. We measured the amplitude of intense blue light–evoked resting membrane potential changes (ΔRMP) in current clamp of opsin-free *gl60j* flies (ΔRMP = 2.85 mV ± 0.3, n = 45) then tested the hypothesis that potassium channel modulation underlies coupling of light-activated CRY to membrane depolarization. To visualize light-evoked lLNv membrane potential changes more clearly, we blocked action potentials in these recordings well past saturation (fig. S3) with the voltage-gated sodium channel blocker tetrodotoxin (TTX), and we assessed the resting membrane potential (RMP) response to light. TTX did not significantly reduce these shifts at 100 nM (ΔRMP = 2.03 ± 0.3, n = 24, P = 0.4 versus control), 500 nM (2.84 ± 0.5, n = 7, P = 1.0 versus control), or 1 μM (2.04 ± 0.4, n = 13, P = 0.7) (Fig. 4F, right). Light-evoked membrane potential changes were, however, significantly decreased by a subsequent application of a voltage-gated and inward rectifier potassium channel blocker cocktail including 10 mM tetraethylammonium (TEA), 2 mM 4-aminopyridine (4-AP), and 2 mM cesium chloride in the presence of 100 nM TTX (ΔRMP = 0.41 mV ± 0.3, n = 34, P < 0.001 versus control). We performed time-matched controls with 100 nM TTX only and found that RMP changes did not differ from control (ΔRMP = 2.77 ± 0.3mV, n = 12, P = 0.9). The K-blocker cocktail also significantly disrupted membrane potential shifts in the absence of TTX (0.78 mV ± 0.2, P < 0.001 versus control, P = 0.9 versus K blockers plus TTX). These results suggest that potassium channel modulation couples to the CRY-mediated lLNv light response.

To determine the precise timing of the CRY-mediated light response, we made recordings in lLNv of *gl60j* mutant flies illuminated with a

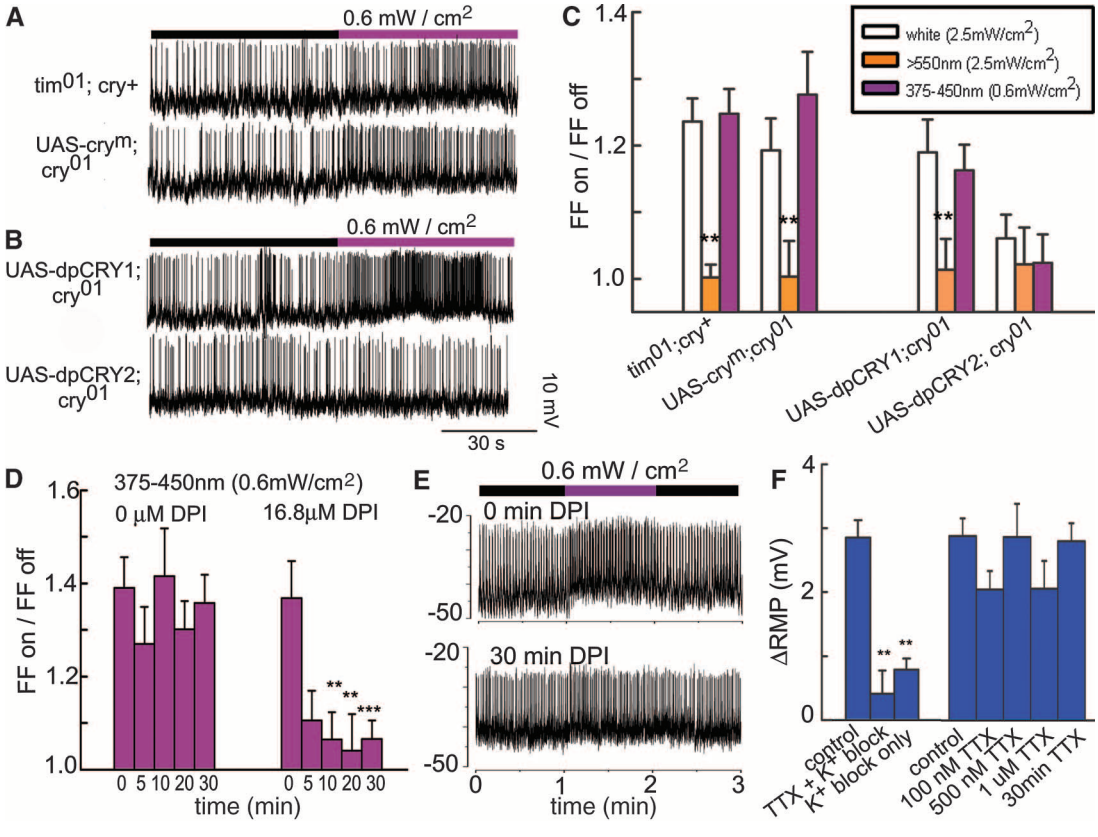

Fig. 4. The CRY-mediated electrophysiological light response membrane depolarization by potassium channel modulation depends on flavin-specific redox reactions rather than TIM interaction. **(A)** Representative recordings of lLNv expressing CRY but *tim* null (tim^{01}, top) and cry^{01} with *pdfGAL4*-driven CRY^{M} (CRY^{M}, bottom). **(B)** Recordings from lLNvs expressing UAS-driven *D. plexippus* dpCRY1 (top) or dpCRY2 exposed to blue-violet (purple bar) after darkness (black bar). **(C)** White and violet light evoke significant responses from each genotype except *dpCRY2 ; cry*^{01}. **(D)** Treatment with the redox inhibitor DPI rapidly attenuates the light response. **(E)** Representative recordings of light-evoked responses in vehicle (top) versus 30 min of DPI treatment (bottom) for the same cell. **(F)** Light-evoked depolarization in lLNv is significantly decreased after treatment with potassium channel blockers TEA, 4-AP, and CsCl in the presence or absence of TTX.

software-triggered blue light source. After ~60 cycles of 4-s blue light pulses from four different lLNvs, records were analyzed in 100-ms bins for spike frequency, 1 s before and after light onset. Although a trend toward more action potentials can be observed during the light pulse, this measurement does not clearly resolve the onset of increased firing in response to light at a millisecond time scale (Fig. 5A). However, application of episodic blue light pulse protocol followed by averaging 120 traces (30 each from four lLNvs) precisely registered by light-on and -off effectively filters the noise from individual records and yields a clear light-evoked RMP response (Fig. 5B). Kinetic analysis reveals that the averaged light-evoked response is best fit with two exponentials with a fast component (τ = 105 ms) and a slower component (τ = 1.07 s). Similarly, the averaged return to the baseline "dark" RMP is also best fit with two exponentials (fast, τ = 106 ms; slow, τ = 1.27 s). Notably, the fast components of the on and off response are nearly identical. The speed of the on response (≈100 ms) is within an order of magnitude of that of classical opsin-based phototransduction (*22*), suggesting that coupling light-activated CRY to depolarization may be diffusion-limited and require intermediate steps. Figure 5C shows individual records that contributed to the averages shown in Fig. 5, either A or B. Although most recordings show an appreciable rise in RMP (sweeps 2 to 5) within 50 ms after lights on (indicated by the arrow and blue shading), increases in firing frequency were difficult to resolve in time bins of this length. Taken together, the results in Figs. 4 and 5 are consistent with the idea that light-activated CRY couples to membrane depolarization via potassium channel modulation.

The white light intensity levels used to characterize the lLNv electrophysiological light response, although five times higher than intensities necessary to induce CRY-TIM interaction, correspond to natural light levels typically observed in the early to mid-morning on a clear day, consistent with recent findings that the lLNv are light-activated morning arousal neurons (*1*–*4*, *23*). We asked what percentage of blue light permeates the cuticle and reaches central brain neurons. We found that the average transmittance through the head cuticle, when compared to phosphate-buffered saline mounting buffer alone, was 55.0% ± 4.3 (n = 7). Importantly, the transmittance through the eye cuticle is similar at 57.2% ± 5.3 (n = 7, P = 0.74 by t test).

CRY mediates a rapid electrophysiological light response that is distinct from classical opsin-based phototransduction. However, the present results do not rule out the possibility that light-activated synaptic inputs from external photoreceptors co-modulate lLNv firing rate in intact animals. Dye-filled individual lLNv cells show extensive arbors in the optic lobe and may reflect sites for external photoreceptor input (*24*). All lines of evidence herein indicate that the CRY-mediated electrophysiological light response is mechanistically distinct from the previously described CRY-TIM interaction. Qualitatively, the CRY-mediated electrophysiological light response bears some resemblance to melanopsin-based light activation of retinal ganglion cells (which underlies circadian entrainment in mammals), whereby light activation leads to increased action potential firing rather than graded potentials found typically in image-forming photoreceptors. The CRY-mediated electrophysiological response appears to exhibit a higher light threshold compared with opsin-based light sensing, which may bias its physiological functions to non–image-forming photosensitive cells.

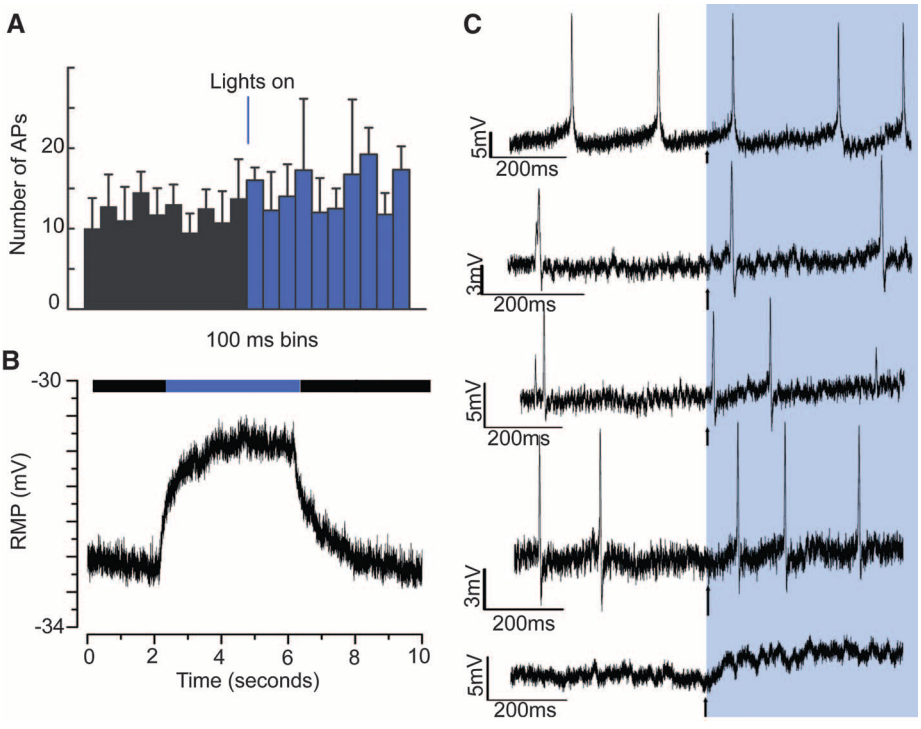

Fig. 5. RMP changes rapidly upon lights on and off. (**A**) Number of spikes in 100-ms bins shown for 1 s before and after the onset of an intense blue light pulse (~35 mW/cm^2), averaged for 60 sweeps from four lLNvs. (**B**) The RMP shows an evoked increase upon triggering of the intense blue light. Trace is an average of 120 sweeps recorded from four lLNvs. The evoked rise and fall were fitted with double exponential functions. (**C**) Individual records that contributed to the averages depicted in (A) and (B). Four of five traces (bottom four traces) show an appreciable rise in RMP within 100 ms, but the increase in FF for the four cells that are firing is apparent only after several hundred milliseconds.

References and Notes

1. V. Sheeba *et al.*, *Curr. Biol.* **18**, 1537 (2008).
2. K. M. Parisky *et al.*, *Neuron* **60**, 672 (2008).
3. Y. Shang, L. C. Griffith, M. Rosbash, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 19587 (2008).
4. V. Sheeba, H. Gu, V. K. Sharma, D. K. O'Dowd, T. C. Holmes, *J. Neurophysiol.* **99**, 976 (2008).
5. C. Helfrich-Förster, M. Stengl, U. Homberg, *Chronobiol. Int.* **15**, 567 (1998).
6. C. Helfrich-Förster, C. Winter, A. Hofbauer, J. C. Hall, R. Stanewsky, *Neuron* **30**, 249 (2001).
7. P. Emery, W. V. So, M. Kaneko, J. C. Hall, M. Rosbash, *Cell* **95**, 669 (1998).
8. K. Koh, X. Zheng, A. Sehgal, *Science* **312**, 1809 (2006).
9. R. Stanewsky *et al.*, *Cell* **95**, 681 (1998).
10. J. Hirsh *et al.*, *Curr. Biol.* **20**, 209 (2010).
11. T. Yoshii, M. Ahmad, C. Helfrich-Förster, *PLoS Biol.* **7**, e1000086 (2009).
12. R. J. Gegear, A. Casselman, S. Waddell, S. M. Reppert, *Nature* **454**, 1014 (2008).
13. R. J. Gegear, L. E. Foley, A. Casselman, S. M. Reppert, *Nature* **463**, 804 (2010).
14. M. N. Nitabach, J. Blau, T. C. Holmes, *Cell* **109**, 485 (2002).
15. G. Cao, M. N. Nitabach, *J. Neurosci.* **28**, 6493 (2008).
16. Materials and methods are available as supporting material on *Science* Online.
17. A. Berndt *et al.*, *J. Biol. Chem.* **282**, 13011 (2007).
18. J. Benito *et al.*, *J. Biol. Rhythms* **23**, 296 (2008).
19. A. Busza, M. Emery-Le, M. Rosbash, P. Emery, *Science* **304**, 1503 (2004).
20. Q. Yuan, D. Metterville, A. D. Briscoe, S. M. Reppert, *Mol. Biol. Evol.* **24**, 948 (2007).
21. B. Collins, E. O. Mazzoni, R. Stanewsky, J. Blau, *Curr. Biol.* **16**, 441 (2006).
22. P. D. Calvert *et al.*, *Nature* **411**, 90 (2001).
23. B. Y. Chung, V. L. Kilman, J. R. Keath, J. L. Pitman, R. Allada, *Curr. Biol.* **19**, 386 (2009).
24. D. Park, L. C. Griffith, *J. Neurophysiol.* **95**, 3955 (2006).

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Supporting Online Material

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References

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